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ABSTRACT:

The 5-year survival rate for BC among US women has increased from 75% during 1974-76 to 85% during 1989-95. Despite such marked improvement, BC is still the leading cause of cancer mortality among women 20 – 59 years of age and the second leading cause of cancer mortality among all women. Disease-free survival after BC treatment is likely predicted by both tumor characteristics and host factors. The clinical and pathologic parameters that have been shown to influence disease prognosis include tumor size, nodal involvement, tumor state, grade, and hormone receptor status, and mitotic index, expression of multi-drug resistance proteins, p53 status, and HER's-2/neu status. Meanwhile, only a few host factors have been identified that impact disease-free or overall survival, particularly those that a patient may engage in to modify or help clinicians to tailor effective and efficient treatment strategy. This proposed study focuses on one-carbon metabolism, a key process for DNA methylation and DNA synthesis. One-carbon metabolism is crucial of BC prognosis because it not only provides methyl group for regulating expression of genes that have prognostic values (e.g. ER, PR, BRCA1, etc.) but also is a primary target for treatment of the disease (e.g. 5-FU, methotrexate, etc.). We propose to utilize the resources of the Long Island Breast Cancer Study Project, a large population-based study consisting of ~1500 BC cases and ~1500 controls. We will examine the dietary intake of one-carbon-related micronutrients/compounds (e.g. folate, methionine, chioline, B vitamins, alcohol, etc) in relation to disease-free and overall survival of BC via the mechanism of promoter hypermethylation (presumably silencing) of the ER, PR, and BRCA1 genes. We will also examine whether functional polymorphisms in one-carbon metabolism may influence survival of BC, either through modifying the efficacy of chemotherapeutic drugs or influencing methylation of prognosis-related genes. Results from this study would help clarify mechanisms of disease progression as well as contribute to the design of a more efficient (genetically tailored) treatment strategy.

Table of Contents

INTRODUCTION

The 5-year survival rate for BC among US women has increased from 75% during 1974-76 to 85% during 1989-95. Despite such marked improvement, BC is still the leading cause of cancer mortality among women 20 – 59 years of age and the second leading cause of cancer mortality among all women. Disease-free survival after BC treatment is likely predicted by both tumor characteristics and host factors. The clinical and pathologic parameters that have been shown to influence disease prognosis include tumor size, nodal involvement, tumor state, grade, hormone receptor status, mitotic index, expression of multi-drug resistance proteins, p53 status, and HER-2/neu status. Meanwhile, only a few host factors have been identified that impact disease-free or overall survival, particularly those that a patient may engage in to modify or help clinicians to tailor effective and efficient treatment strategy. This proposed study focuses on one-carbon metabolism, a key process for DNA methylation and DNA synthesis. One-carbon metabolism is crucial of BC prognosis because it not only provides methyl group for regulating expression of genes that have prognostic values (e.g. *ER, PR, BRCA1,* etc.) but also is a primary target for treatment of the disease (e.g. 5-FU, methotrexate, etc.). We propose to utilize the resources of the Long Island Breast Cancer Study Project, a large population-based study consisting of ~1500 BC cases and ~1500 controls. We will examine the dietary intake of one-carbon-related micronutrients/compounds (e.g. folate, methionine, chioline, B vitamins, alcohol, etc) in relation to disease-free and overall survival of BC *via* the mechanism of promoter hypermethylation (presumably silencing) of the ER, PR, and BRCA1 genes. We will also examine whether functional polymorphisms in one-carbon metabolism may influence survival of BC, either through modifying the efficacy of chemotherapeutic drugs or influencing methylation of prognosis-related genes. Results from this study would help clarify mechanisms of disease progression as well as contribute to the design of a more efficient (genetically tailored) treatment strategy.

BODY

Task 1. **To genotype polymorphisms in one-carbon-metabolizing genes on 1087 BC cases (Months 1- 24)**

Genotyping has been completed as reported previously.

Task 2. **Determine the promoter methylation patterns on *ER, PR,* and *BRCA1* genes from ~960 BC tissues (Months 1-30)**

a. DNA extraction from ~960 tumor blocks.
b. Set up and validate methylation assay on *ER, PR* and *BRCA1* genes using a real-time quantitative methylation-specific PCR method.

These tasks have been completed as reported previously.

c. Ascertain methylation patterns of *ER, PR,* and *BRCA1* from 960 tumor tissues.

By using the methylation-specific PCR (MS-PCR) assay, we determined the methylation status of three genes, ERα, PRβ and BRCA1, for ~850 breast cancer samples. We detected 383(44.8%) promoter hypermethylation for ERα; 102(11.9%) for PRβ; 504(59.0%) for BRCA1, respectively.

d. Data entry.

All genotype and gene promoter methylation status data have been entered into our database and are ready to analyze.

Task 3. Data analyses (Months 25-36)

a. Study associations of dietary methyl content and overall survival.

Some descriptive statistical analysis has been reported in previous annual report. The Kaplan-Meier method and the log-rank test were used for univariate survival analysis for dietary B vitamin intake. The Cox Proportional-Hazards regression was used to estimate hazard ratio and 95% confidence interval (95% CI) in the age-adjusted regression models for both all-cause and breast cancer-specific mortality.
The relationship between base-line dietary B vitamin intake and overall survival was summarized in **Table 1.** Intake of vitamin B1 and B3 were found to associate with overall survival in this population. Compared to the lowest intake quintile, cases in the highest or second highest intake quintile have about 50% lower risk of death, which is statistically significant. These relationships were in dose-dependent manner (p,trend = 0.01 for both vitamin B1 and B3).

Table 1 Association between B vitamin intake and overall survival

Variable	Quintiles of intake					
	Q1	**Q2**	**Q3**	**Q4**	**Q5**	**P, trend**
Dietary folate						
Range(µg/d)	<159	159-216	216-279	279-356	>356	
HR(95% CI)	1.00(ref)	0.97(0.62-1.50)	0.88(0.56-1.38)	0.76(0.46-1.25)	0.81(0.47-1.39)	0.28
Total folate						
Range(µg/d)	<208	208-330	330-561	561-722	>722	
HR(95% CI)	1.00(ref)	0.78(0.50-1.20)	0.70(0.44-1.13)	0.93(0.60-1.42)	0.85(0.54-1.35)	0.80
Vitamin B1						
Range(mg/d)	<0.72	0.72-0.95	0.95-1.16	1.16-1.48	>1.48	
HR(95% CI)	1.00(ref)	0.81(0.53-1.24)	0.67(0.41-1.07)	0.50(0.29-0.86)	0.52(0.28-0.98)	0.01
Vitamin B2						
Range(mg/d)	<0.95	0.95-1.30	1.30-1.62	1.62-2.12	>2.12	
HR(95% CI)	1.00(ref)	0.97(0.63-1.50)	0.68(0.40-1.13)	0.92(0.56-1.54)	0.79(0.43-1.47)	0.47
Vitamin B3						
Range(mg/d)	<9.8	9.8-12.9	12.9-15.7	15.7-19.9	>19.9	
HR(95% CI)	1.00(ref)	0.78(0.51-1.19)	0.67(0.41-1.07)	0.48(0.28-0.81)	0.82(0.28-0.98)	0.01
Vitamin B6						
Range(mg/d)	<0.84	0.84-1.15	1.15-1.42	1.42-1.84	>1.84	
HR(95% CI)	1.00(ref)	0.71(0.46-1.10)	0.63(0.39-1.03)	0.67(0.40-1.10)	0.77(0.44-1.35)	0.35

b. Study associations of one-carbon polymorphisms and overall survival.

Follow the crude analysis of one-carbon polymorphism in relation to survival, we built multivariate model and examined the association for breast cancer specific survival. Results from the multivariate model were similar to the age-adjusted model..
Variant allele of two polymorphisms, *MTHFR677* and *BHMT*, were statistically significantly associated with better survival. The *MTHFR677* T allele carriers have 31% lower risk of death than patients with the *MTHFR677* CC genotype (HR and 95% CI: 0.69(0.49-0.98)). A allele carries *BHMT* polymorphism have 30% lower risk of death than those with the *BHMT* GG genotype (HR and 95% CI: 0.70(0.50-1.00)). Two other SNPs, *MTR2756* G and *cSHMT1420* T alleles were associated with better survival with borderline significance.

Models were run using breast cancer-specific mortality as outcome (data not shown) and the results were similar to those for all-cause mortality as outcome.
In **Table 2**, results of subgroup analysis were summarized. Cases were divided into two groups: ER+PR+ cases and otherwise (ER+/PR-, ER-/PR+, ER-/PR-) due to the relative infrequency. The association of one-carbon metabolism polymorphisms and overall survival was different by ER/PR status for several polymorphisms, i.e. *MTHFR C677T* (p,int = 0.05). ER/PR status significantly modified the effect of *MTHFR677* on survival with the T allele was associated with worse survival among ER+/PR+ cases. However, the point estimate was not significant.

Table 2 Stratified analysis of associations of one-carbon polymorphisms with overall survival

		ER/PR status		**Chemotheray**	
		+	**-**	**yes**	**no**
Gene	**Genotype**	**HR**	**HR**	**HR**	**HR**
MTHFR	0	1.00(ref)	1.00(ref)	1.00(ref)	1.00(ref)
(C677T)	1	1.47	0.61	1.13	0.86
	P,int	**0.05**		**0.55**	
MTHFR	0	1.00(ref)	1.00(ref)	1.00(ref)	1.00(ref)
(A1208C)	1	1.27	0.66	1.22	0.91
	P,int	**0.13**		**0.64**	
TSTR	0	1.00(ref)	1.00(ref)	1.00(ref)	1.00(ref)
(5'-UTR)	1	0.59	1.06	1.59	0.51
	P,int	**0.21**		**0.13**	
DHRF	0	1.00(ref)	1.00(ref)	1.00(ref)	1.00(ref)
(19bp del)	1	1.51	0.68	0.82	1.34
	P,int	**0.10**		**0.61**	
MTR	0	1.00(ref)	1.00(ref)	1.00(ref)	1.00(ref)
(A2756G)	1	0.66	0.84	0.83	1.33
	P,int	**0.64**		**0.5**	
MTRR	0	1.00(ref)	1.00(ref)	1.00(ref)	1.00(ref)
(A66G)	1	0.59	0.70	0.60	0.63
	P,int	**0.72**		**1**	
BHMT	0	1.00(ref)	1.00(ref)	1.00(ref)	1.00(ref)
(G742A)	1	1.21	0.61	0.62	0.66
	P,int	**0.12**		**0.78**	
RFC1	0	1.00(ref)	1.00(ref)	1.00(ref)	1.00(ref)
(A80G)	1	1.28	1.12	0.99	0.81
	P,int	**0.78**		**0.97**	
cSHMT	0	1.00(ref)	1.00(ref)	1.00(ref)	1.00(ref)
(C1420T)	1	1.20	0.58	0.79	0.77
	P,int	**0.09**		**0.91**	

c. Study associations of one-carbon metabolism (diet and polymorphism) and methylation patterns of *ER, PR* and *BRCA1*.

We explored the association of dietary and total folate intake and gene promotor methylation status. Only PR-beta methylation status was correlated with total folate intake. Compared to the lower intake group, the higher intake group has higher percentage of methylation.
In the crude analyses, polymorphisms of one-carbon metabolism pathway do not predict methylation status of these three genes. However, cases with 2R/2R genotype of the TS gene are more likely to have methylated ERα (OR:1.68; 95%CI: 1.06-2.65).

d. Study associations of methylation pattern and overall survival.

The survival does not differ by the methylation status of ERα, PRβ. However, BRCA1 promoter methylation was associated with increased overall mortality (age-adjusted HR 1.49; 95% CI: 1.02-2.18) as well as breast cancer-specific mortality (age-adjusted HR 1.71; 95% CI: 1.05-2.78).

e. Study survival relationship by treatment regimen (i.e. chemotherapy vs. no chemotherapy).

Cases were divided into two groups by whether received chemotherapy. This analysis was carried out to explore the potential modifying effect of one-carbon gene polymorphisms on chemotherapy response in relation to breast cancer survival. Results were summarized in **Table 2.** The association of one-carbon metabolism polymorphism and overall survival does not differ by whether received chemotherapy or not.

f. Manuscript preparation.

See publication list below.

KEY RESEARCH ACCOMPLISHMENTS

1. We have completed the promoter methylation status detection for three genes, namely ERα, PRβ and BRCA1.
2. All data from lab measurement have been entered into our database for analysis.
3. Preliminary analysis were done for association between dietary methyl content and overall survival; one-carbon polymorphisms and overall survival; one-carbon metabolism and gene promoter methylation patterns; gene promoter methylation pattern and overall survival; and one-carbon polymorphisms and treatment regimen in relation to survival.

CONCLUSIONS

We found two one-carbon polymorphisms, namely, *MTHFR C677T* and *BHMT G742A*, were statistically significantly associate with overall survival which indicates that one-carbon may play important role in disease prognosis. Gene promoter methylation status was not found to be significant associated survival in this population.

REPORTABLE OUTCOMES

Manuscripts:

1) Xu X, Gammon MD, Wetmur JG, Rao M, Gaudet MM, Teitelbaum SL, Britton JA, Neugut AI, Santella RM, Chen J. A functional 19-base pair deletion polymorphism of dihydrofolate reductase (DHFR) and risk of breast cancer in multivitamin users. *Am J Clin Nutr*. 85(4): 1098-1102.

2) Xu X, Gammon MD, Zhang H, Wetmur JG, Rao M, Teitelbaum SL, Britton JA, Neugut AI, Santella RM, Chen J. Polymorphisms of One-carbon Metabolizing Genes and Risk of Breast Cancer in a Population-based Study. *Carcinogenesis*. 2007 Jul;28(7):1504-9. Epub 2007 Mar 19

3) Xu X, Gammon MD, Zeisel SH, Lee YL, Wetmur JG, Teitelbaum SL, Bradshaw PT, Neugut AI, Santella RM, Chen J. Choline metabolism and risk of breast cancer in a population-based study. *FASEB J*. 2008 22: 2045-2052. Epub 2008 Jan 29

4) Xu X, Gammon MD, Zhang Y, Bestor TH, Zeisel SH, Wetmur JG, Wallenstein S,
Bradshaw PT, Garbowski G, Teitelbaum SL, Neugut AI, Santella RM, Chen J. BRCA1 promoter methylation is associated with increased mortality among women with breast cancer. *Breast Cancer Res Treat*. 2008 Jun 3. [Epub ahead of print]

5) Xu X, Gammon MD, Wetmur JG, Bradshaw PT, Teitelbaum SL, Neugut AI, Santella RM, Chen J. B-vitamin Intake, One-carbon Metabolism and Survival among a Population-based Study of Women with Breast Cancer. *Cancer Epidemiol Biomarkers Prev*. (in press)

Meeting Abstract

(1) "A 19bp Deletion Polymorphism of A Folate-Metabolizing Gene, Dihydrofolate Reductase(DHFR),and Risk of Breast Cancer" - Department of Defense Breast Cancer Research Program Meeting, The 4th Era of Hope in Philadelphia, PA, June 8-11,2005

(2) "Functionality of the DHFR 19bp Deletion Polymorphism and its Association with Breast Cancer Risk among Multivitamin Users" - American Association for Cancer Research 97th Annual Meeting, Washington DC, April 1-5,2006

(3) "Polymorphisms of One-carbon Metabolizing Genes and Risk of Breast Cancer in a Population-based Study" - American Association for Cancer Research 98th Annual Meeting, Los Angeles, CA, April 14-18, 2007

(4) "Choline Metabolism and Risk of Breast Cancer in a Population-based Study" - American Association for Cancer Research 99th Annual Meeting, San Diego, CA, April 12-16, 2008

(5) "BRCA1 Promoter Methylation is Associated with Increased Mortality among Women with Breast Cancer" - American Association for Cancer Research 99th Annual Meeting, San Diego, CA, April 12-16, 2008

APPENDICES

None